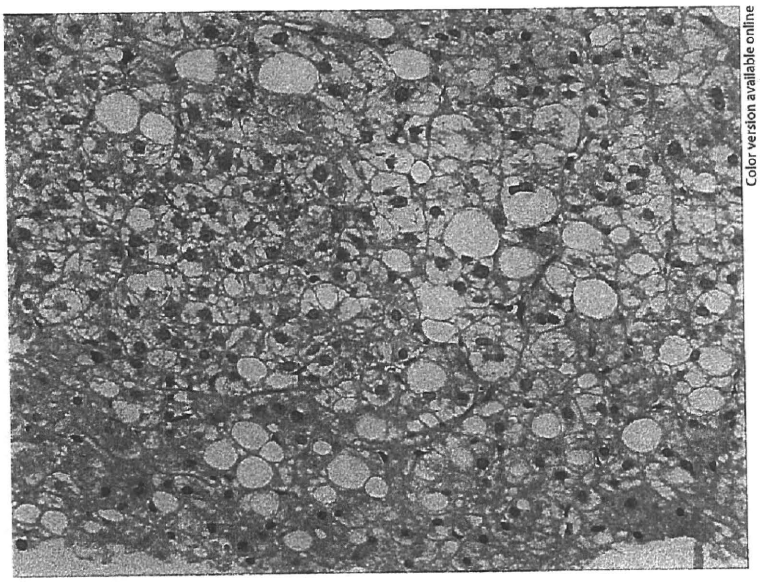

Fig. 5. A needle biopsy of the nodule was done which revealed a well-differentiated HCC. HE. 200×.

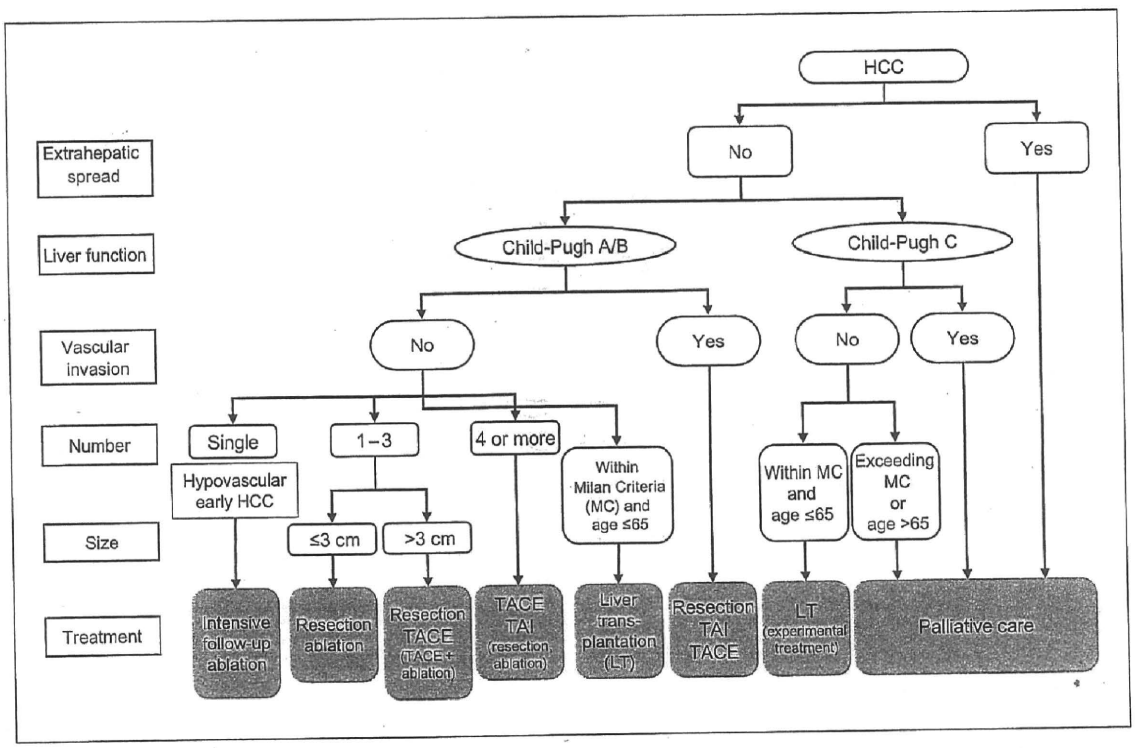

Fig. 6. Treatment algorithm for HCC (JSH consensus-based 2007).

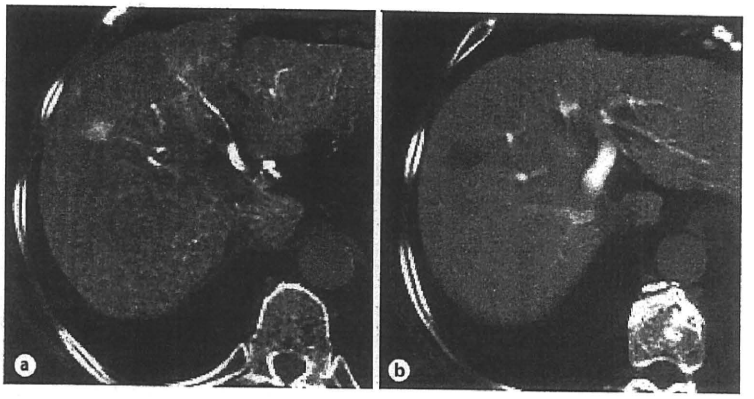

Fig. 7. A representative case with a hypervascular nodule by CTHA in segment 8 with a diameter of 1.8 cm (**a**). This nodule became a low-density area by CTAP (**b**). This patient has good liver function classified as Child-Pugh A.

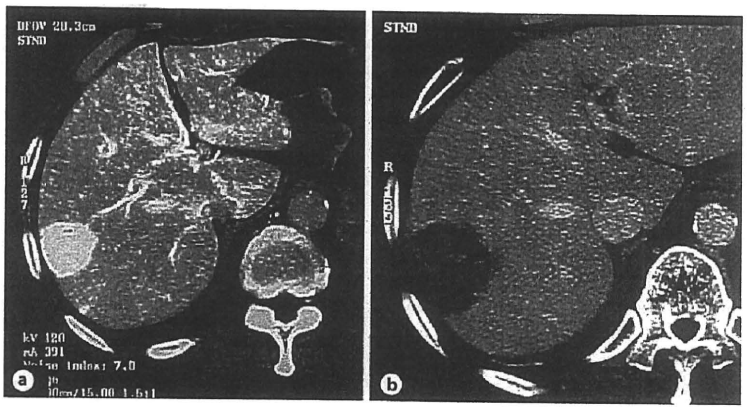

Fig. 8. A representative case with a hypervascular nodule by CTHA in segment 7 with a diameter of 3.0 cm (**a**). Locally complete curative necrosis was achieved by RFA (**b**).

Statement 4

Which treatment do you choose for the patient having a single HCC nodule <2 cm with Child-Pugh A?

A typical case of a 62-year-old male is shown in figure 7. A hypervascular small nodule was observed by CTHA at segment 8 with a diameter of 1.8 cm, which became a low-intensity area by CTAP. His liver function was classified as Child-Pugh A.

38% of the participants chose surgical resection, but 62% chose RFA. At the JSH consensus meeting, 44% of the participants chose surgical resection, but 56% chose RFA. When only surgeons were asked the same question, 80% of them chose surgical resection, but 20% chose RFA. This question was asked to only physicians at the JSH consensus meeting, and 32% chose surgical resection, and 68% chose RFA.

Overall survival was compared after surgical resection with RFA [8], in which no apparent difference was observed between the two groups. Thereafter, several reports including a large number of patients with HCC compared the survival or recurrence, but they are not randomized [9–11]. This is an important issue that needs to be clarified. Thus, randomized controlled trials are necessary including a large number of patients to clarify which treatment is superior between surgical resection and RFA.

Statement 5

Which treatment do you choose for a patient having a single HCC nodule <3 cm with Child-Pugh A?

A representative case of a 75-year-old male is shown in figure 8a. He had a single hypervascular nodule defined by CTHA at segment 7 with a diameter of 3 cm. His liver

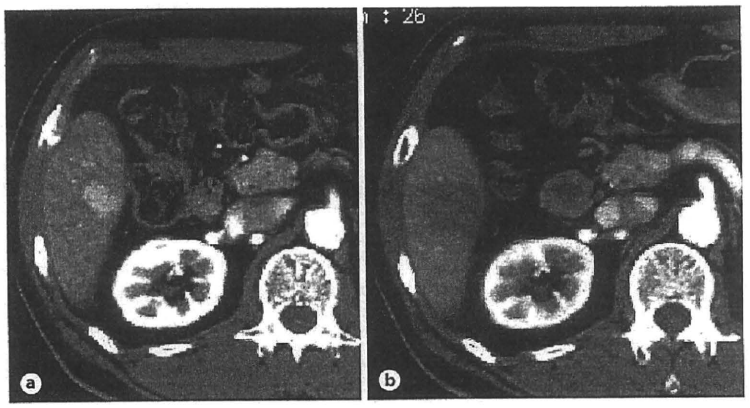

Fig. 9. A typical case with a single hypervascular HCC nodule in segment 6 with a diameter of 1.4 cm. He has a good liver function of Child-Pugh A (**a**). He was treated by RFA alone, and complete necrosis was obtained (**b**).

function was well preserved and he was classified as Child-Pugh A. The participants were asked this question. 74% of the participants chose surgical resection, but 26% chose RFA by the ILCA. At the JSH consensus meeting, 80% of the participants chose surgical resection, but 20% chose RFA as the first-line treatment.

Interestingly enough, most of the participants chose surgical resection when the nodule was as large as 3 cm. This hypervascular HCC nodule was treated by percutaneous RFA, and locally complete curative necrosis was obtained (fig. 8b).

Statement 6

RFA should be done after TACE to the hypervascular HCC nodule with a diameter of 2 cm.

A typical 62-year-old male with a 1.6-cm single hypervascular HCC nodule in segment 6 is shown in figure 9a. He has a good liver function with Child-Pugh A.

This statement was agreed on by 36% of the participants at the ILCA consensus meeting, but 64% disagreed. However, at the JSH consensus meeting, 51% of the participants agreed with this statement, but 49% disagreed.

This hypervascular HCC nodule was completely ablated by RFA alone (fig. 9b). It has been reported that TACE before RFA increased the ablated area, suggesting that overall survival will improve [12–14]; however, TACE may increase the adverse events by RFA. Whether TACE before RFA is beneficial for the patients should be examined by analyzing the overall survival of patients and comparing them to receiving TACE before RFA or without TACE before RFA.

Statement 7

Do you prescribe sorafenib as the first-line treatment option for the patients with advanced HCC in whom surgical resection, RFA or TACE is not indicated?

As sorafenib was approved in Japan in May 2009 [15], only a few hepatologists have experienced prescribing the medicine. Its usefulness after TACE in patients with advanced HCC is under investigation in the USA and Japan [16]. It will be included in the therapeutic algorithm for HCC, but it is still unclear to hepatologists to which patients the medicine should be prescribed.

At the ILCA consensus meeting, 61% of the participants agreed with this statement, but 30% disagreed. 9% of them did not have any opinion on the statement because they have no experience with sorafenib. At the JSH consensus meeting, 35% of the participants agreed with this statement, but 29% disagreed. 36% of the participants did not have any opinion because they have no experience with sorafenib.

The best indication for sorafenib should be investigated in the near future [17].

Statement 8

Overall survival should be the endpoint for the assessment of efficacy comparing ablation with surgical resection.

The recurrence rate after surgical resection or RFA was reported including a large number of patients, and the incidence of intrahepatic recurrence was higher after RFA than surgical resection [11]. However, overall survival was not different between the two groups. Thus, the problem is how to evaluate the outcome of surgical resection and RFA, and this question was proposed by hepatologists.

This statement was agreed on by 89% of the participants at the ILCA consensus meeting, but 11% disagreed with this statement. At the JSH consensus meeting, 84% of the participants agreed with this statement, and 16% disagreed. The outcome should be evaluated by both overall survival and incidence of recurrence.

Acknowledgement

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Disclosure Statement

The author declares that he has no financial conflict of interest.

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Original Article

Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy

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Aim: Hepatic steatosis is linked to development of hepatocellular carcinoma (HCC) in non-viral liver disease such as non-alcoholic steatohepatitis. The present study aimed to assess whether hepatic steatosis is associated with the development of HCC in chronic hepatitis C.

Methods: We studied a retrospective cohort of 1279 patients with chronic hepatitis C who received interferon (IFN) therapy between 1994 and 2005 at a single regional hospital in Japan. Of these patients, 393 had a sustained virological response (SVR) and 886 had non-SVR to IFN therapy. After IFN therapy, these patients were screened for development of HCC every 6 months. The average period of observation was 4.5 years.

Results: HCC developed in 68 patients. The annual incidence of HCC was 2.73% for patients with a steatosis grade of 10% or greater and 0.69% for patients with a steatosis grade of 0–9%. On multivariate analysis, higher grade of steatosis was a significant risk factor for HCC independent of older age, male sex, higher body mass index (BMI), advanced fibrosis stage and non-SVR to IFN therapy. The adjusted risk ratio of hepatic steatosis was 3.04 (confidence interval 1.82–5.06, $P < 0.0001$), which was higher than that of older age (1.09), male sex (2.12), non-SVR to IFN (2.43) and higher BMI (1.69).

Conclusion: Hepatic steatosis is a significant risk factor for development of HCC in chronic hepatitis C independent of other known risk factors, which suggest the possibility that amelioration of hepatic steatosis may prevent hepatocarcinogenesis.

Key words: hepatocellular carcinoma, interferon, steatosis, virological response.

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers worldwide and its incidence has been increasing. This recent increase in HCC incidence may likely be attributed to the higher prevalence of non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV) infection.[1]

Non-alcoholic fatty liver disease is characterized by hepatic steatosis with or without inflammation in the absence of excessive alcohol consumption. Several studies have indicated the etiological association between NAFLD and development of HCC.[2–4] Other studies have shown that obesity or diabetes, a common etiology of non-alcoholic hepatic steatosis, is associated with development of HCC.[5–7] Although the mechanism of carcinogenesis in NAFLD has not been determined, an animal model showed that obesity-related hepatic steatosis leads to the development of hepatic

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hyperplasia, suggesting the possibility that hepatic steatosis is a pre-malignant condition.[8]

Another important etiological agent for HCC is HCV infection. Because steatosis is a common pathological feature of HCV-infected patients,[9] the important question is whether steatosis influences the progression of liver disease in hepatitis C, by analogy with NAFLD. Several studies, including ours[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis.[11–15] The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed[16] and was confirmed in two studies[17,18] while another study failed to show such an association.[19] The present study was conducted to analyze the association between hepatic steatosis and development of HCC in a large cohort of chronic hepatitis C patients, which enabled to adjust for known risk factors for HCC.

METHODS

Patients

A TOTAL OF 1437 chronic hepatitis C patients were treated with interferon (IFN) at Musashino Red Cross Hospital between October 1994 and October 2005. Among them, 1279 patients who fulfilled the following inclusion criteria were enrolled in this study: (i) positive for HCV RNA by reverse-transcription polymerase chain reaction before IFN therapy; (ii) absence of other causes of liver disease, such as co-infection with hepatitis B virus, autoimmune hepatitis or primary biliary cirrhosis; (iii) had undergone liver biopsy within the 12 months prior to IFN treatment; (iv) were followed for more than 1 year after the completion of IFN therapy; and (v) absence of HCC during and within 1 year after the completion of therapy. A total of 158 patients were excluded: two patients who were positive for hepatitis B surface antigen, 97 patients lacking liver biopsy, 53 patients with less than 1 year's duration of follow up, and six patients who developed HCC within 1 year of the completion of IFN therapy. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committee.

Patients were followed up by regular visits to our hospital every 1–3 months. Six patients died of liver-unrelated disease (two patients with gastric cancer and one patient each with lung cancer, colon cancer, pancreatic cancer and leukemia). There were 122 patients who were lost to follow up because of relocation. We included their data in the analysis, censored at the time of their last visit. The start of follow up was defined as the date of completion of first IFN therapy and the end of follow up was defined as the date of diagnosis of HCC or the date of the last visit. The average period of follow up was 4.5 years.

Clinical characteristics and laboratory data were collected at the most recent time point before liver biopsy. Diabetes mellitus was diagnosed based on a fasting plasma glucose concentration that exceeded 126 mg/dL, a casual plasma glucose concentration that exceeded 200 mg/dL, or the need for insulin or oral antihyperglycemic drugs. Information regarding alcohol consumption was obtained through an interview. Body mass index (BMI) was calculated using the following formula: weight in kilograms/height in meters squared. The baseline clinical features of patients at enrollment are summarized in Table 1.

Histological examination

Liver biopsy specimens were obtained from all patients before therapy. The median length of liver biopsy specimens was 13 mm (range 10–42 mm) and median number of portal tracts was 11 (range 4–30). Histological findings were re-evaluated recently by three independent pathologists who were blinded to the clinical details to ensure consistency over time. Fibrosis and activity were scored according to the METAVIR scoring system.[20] Fibrosis was staged on a scale of 0–4: F0 (no fibrosis); F1 (mild fibrosis: portal fibrosis without septa); F2 (moderate fibrosis: few septa); F3 (severe fibrosis: numerous septa without cirrhosis); and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity); A1 (mild activity); A2 (moderate activity); and A3 (severe activity). Percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis and graded on a scale of 0%, 1–9%, 10–29% and 30% or greater as reported previously.[10] All three pathologists assigned the same scale in 85% of cases for fibrosis staging, 87% for inflammation grading and 95% for steatosis grading. If there was discordance, the scores assigned by two pathologists were used for the analysis.

Screening for HCC

At enrollment, no patient had HCC or any suspicious lesion on abdominal ultrasonography or computed tomography. Patients were examined for HCC by abdominal ultrasonography or computed tomography at least every 6 months. Suspicious lesions were examined further by a triphasic contrast-enhanced computerized tomography or magnetic resonance imaging,

Table 1 Clinical characteristics of patients

Male, *n* (%)	643 (50%)
Age (years)	54.2 ± 11.9
BMI (kg/m^2)	23.4 ± 3.1
Alcohol consumption ≥20 g/day, *n* (%)	44 (3%)
Diabetes Mellitus, *n* (%)	197 (15%)
AST level (IU/L)	68.9 ± 45.3
ALT level (IU/L)	92.9 ± 75.9
GGT level (IU/L)	41.2 ± 38.2
Platelet count (×10^{10}/L)	16.4 ± 5.2
HCV genotype, *n* (%)	
1b	873 (68.2%)
2a	236 (18.4%)
2b	139 (10.9%)
3	2 (0.2%)
Not determined	29 (2.3%)
Histological findings	
Grade of activity, *n* (%)	
A0	154 (12%)
A1	574 (45%)
A2	441 (34%)
A3	110 (9%)
Stage of fibrosis, *n* (%)	
F0	24 (2%)
F1	591 (46%)
F2	378 (30%)
F3	242 (19%)
F4	44 (3%)
Grade of steatosis, *n* (%)	
0%	384 (30%)
1–9%	543 (42%)
10–29%	215 (17%)
≥30%	137 (11%)
SVR to interferon therapy, *n* (%)	393 (31%)
Development of HCC, *n* (%)	68 (5%)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.

angiography or tumor biopsy to confirm the diagnosis. Diagnostic criteria of HCC on radiological findings were hyper-vascularity at angiography or hyper-attenuation at triphasic contrast-enhanced computerized tomography or magnetic resonance imaging during the hepatic arterial phase.

Statistical analysis

The SPSS software package ver. 15.0 was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. The time for the development of HCC was defined as the time from the completion of IFN therapy to the time of diagnosis. Annual incidence of HCC was calculated using the person-years method. Effect of hepatic steatosis on time to development of HCC was analyzed by the Kaplan–Meier method and log-rank test, after stratification by age, sex, BMI, degree of fibrosis and response to IFN therapy, as well as multivariate analysis using Cox proportional hazards regression analysis. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Background factors for steatosis

PATIENTS WITH A steatosis grade of 10% or greater were older (53.6 ± 12.6 vs 56.0 ± 9.8, $P = 0.001$), had a higher BMI (23.0 ± 3.0 vs 24.6 ± 3.3, $P < 0.0001$), higher frequency of diabetes (12% vs 24%, $P < 0.0001$), higher serum levels of aspartate aminotransferase (AST) (66 ± 46 vs 75 ± 43, $P = 0.002$), γ-glutamyltransferase (GGT) (37 ± 52 vs 52 ± 33, $P < 0.0001$), total cholesterol (173 ± 32 vs 179 ± 33, $P = 0.005$), triglycerides (123 ± 56 vs 145 ± 68, $P < 0.0001$), and a lower serum level of albumin (4.2 ± 0.3 vs 4.1 ± 0.3, $P = 0.005$) and lower platelet counts (16.6 ± 5.2 vs 15.7 ± 5.1, $P = 0.007$). Histological grade of activity (A2–3: 39% vs 54%, $P < 0.0001$), and stage of fibrosis (F3–4: 18% vs 34%, $P < 0.0001$) were higher. The proportion of non-sustained virological response (SVR) to IFN also was higher (35% vs 19%, $P < 0.0001$). These results indicate that hepatic steatosis in hepatitis C is related to metabolic factors and associated with other risk factors for the development of HCC such as older age, advanced stage of fibrosis, and non-SVR to IFN therapy.

Factors associated with the development of HCC

Hepatocellular carcinoma developed in 68 patients during follow up. An overall annual incidence of HCC development was 1.19% by person-years. The annual incidence of HCC development by person-years was higher in patients with higher grade of steatosis: 0.45% for patients without steatosis, 0.78% for patients with 1–9% of steatosis, 2.30% for patients with 10–29% of steatosis, and 3.56% for patients with 30% of steatosis. The relative risk of hepatic steatosis (grade of ≥10%) for HCC development was 4.39 (95% confidence interval 2.66–7.26, $P < 0.0001$). The difference remained significant, even after stratification for other risk factors such as IFN therapy, stage of fibrosis, age, sex and BMI (Fig. 1). When analyzed by the multivariate Cox proportional hazards regression method, a higher grade of steatosis,

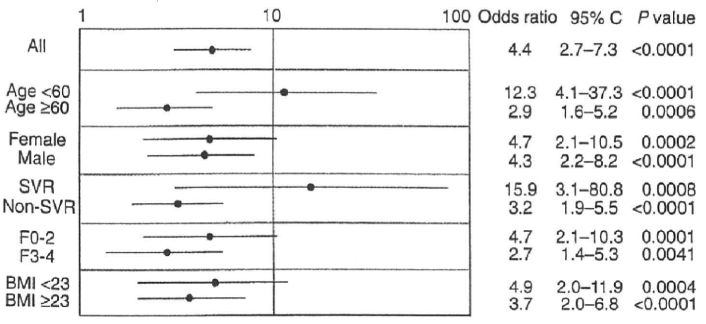


Figure 1 Relative risk differences of hepatocellular carcinoma (HCC) among patients with and without steatosis. The relative risk of hepatic steatosis (grade ≥10%) for HCC development was analyzed, after stratification for other risk factors such as interferon (IFN) therapy, stage of fibrosis, age, sex and body mass index (BMI). SVR, sustained virological response.

older age, male sex, higher BMI, an advanced stage of fibrosis and non-SVR to IFN therapy were independent risk factors associated with the development of HCC (Table 2). The adjusted risk ratio of hepatic steatosis was 3.04 (95% confidence interval 1.82–5.06, $P < 0.0001$). The presence of diabetes and consumption of ethanol were not significant. Figure 2(a) shows the Kaplan–Meier curve of the time to development of HCC in the entire cohort. The cumulative incidence of HCC was significantly higher with hepatic steatosis of 10% or greater. To adjust for other risk factors, patients were stratified according to response to IFN therapy, stage of fibrosis, age, sex and BMI. The difference remained significant, even after stratification for these confounding factors (Fig. 2b–f). Three patients died after the development of HCC. All were over 60 years old, and had significant steatosis. The impact of hepatic steatosis on the survival rate could not be analyzed due to the small number of death.

DISCUSSION

IN THIS STUDY, we have shown that the presence of significant steatosis is an independent risk factor for the development of HCC in chronic hepatitis C. Our study involved the largest number of patients, compared to previous reports, and this enabled us to adjust for other known risk factors for HCC. The impact of steatosis on HCC development remained significant even after adjusting for other risk factors such as older age, male sex, higher BMI, advanced fibrosis and non-SVR to IFN therapy. These findings indicate the need of intensive surveillance for HCC in patients with significant steatosis and provide an argument for therapeutic interventions aimed at reducing steatosis, in order to reduce the risk of HCC.

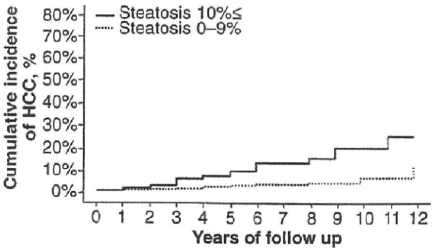
The association between hepatic steatosis and the development of HCC in chronic hepatitis C has been proposed and the possible mechanism has been discussed.[16] There are several cohort studies on this topic but their results are conflicting. The first report included 20 patients with SVR to IFN, 51 patients with non-SVR to IFN and 90 patients who did not receive IFN therapy.[17] In this cohort of 161 patients, older age, absence of IFN therapy, cirrhosis and steatosis were associated with HCC development. Another study involved 25 patients with HCC and an equal number of patients who did not develop HCC, matched for

Table 2 Multivariate analysis of risk factors for hepatocellular carcinoma

Predictor		Odds ratio (95% CI)	*P*-value
Age	By every 10 years	1.09 (1.05–1.13)	<0.0001
Sex	Male vs female	2.12 (1.28–3.51)	0.004
Stage of fibrosis	F3–4 vs F0–2	4.30 (2.59–7.14)	<0.0001
Grade of steatosis	≥10% vs <10%	3.04 (1.82–5.06)	<0.0001
Response to IFN	Non-SVR vs SVR	2.43 (1.13–5.23)	0.023
Diabetes	Present vs absent	0.75 (0.42–1.33)	0.319
Ethanol consumption (g/day)	≥20 vs <20	0.50 (0.07–3.60)	0.478
BMI (kg/m2)	≥23 vs <23	1.69 (1.02–2.86)	0.043

BMI, body mass index; CI, confidence interval; IFN, interferon; SVR, sustained virological response.

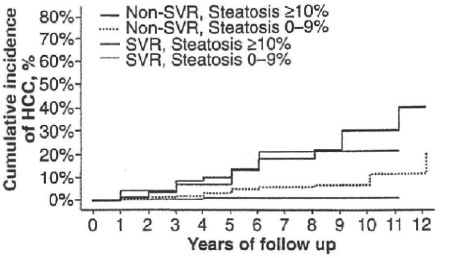
(a)



Number of patients at risk

Steatosis 0–9%	927	824	620	503	320	227	161	117	77	49	27	10
Steatosis ≥10%	352	271	207	157	113	83	54	48	32	17	9	1

(b)



Number of patients at risk

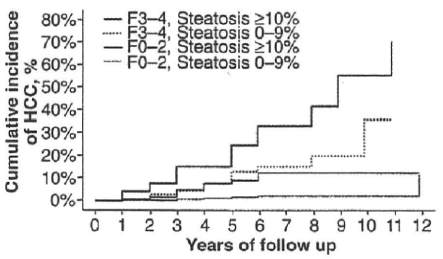
SVR

Steatosis 0–9%	326	254	204	153	81	55	33	21	15	10	5	0
Steatosis ≥10%	67	50	34	22	14	10	4	4	4	2	2	0

Non-SVR

Steatosis 0–9%	601	507	416	350	239	172	128	96	62	39	22	10
Steatosis ≥10%	285	221	173	135	99	73	50	44	28	15	7	1

(c)



Number of patients at risk

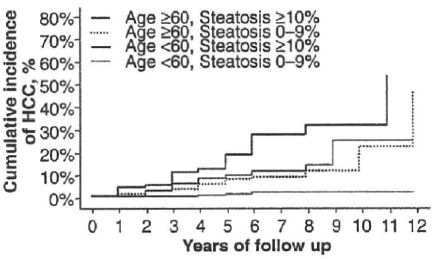
F0–2

Steatosis 0–9%	759	623	509	415	266	188	137	99	64	39	25	10
Steatosis ≥10%	234	190	146	107	77	55	37	32	19	11	6	1

F3–4

Steatosis 0–9%	118	81	61	50	36	28	17	16	13	6	3	0
Steatosis ≥10%	168	138	111	88	54	39	23	18	13	10	2	0

(d)



Number of patients at risk

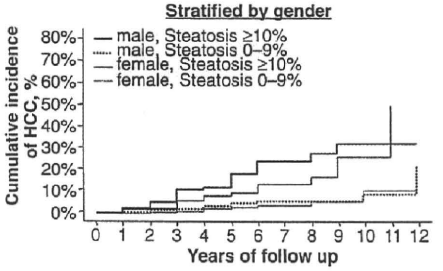
Age <60

Steatosis 0–9%	549	457	367	298	188	148	111	83	53	33	19	7
Steatosis ≥10%	193	154	111	83	61	48	34	31	23	12	6	1

Age ≥60

Steatosis 0–9%	378	304	253	205	132	79	50	34	24	16	8	3
Steatosis ≥10%	159	117	96	74	52	35	20	17	9	5	3	0

(e)



Number of patients at risk

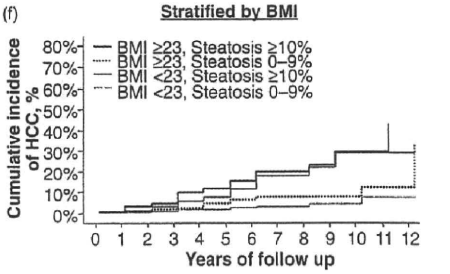
Male

Steatosis 0–9%	470	389	319	265	169	126	90	65	46	30	17	7
Steatosis ≥10%	173	134	98	73	54	40	21	21	15	8	6	1

Female

Steatosis 0–9%	457	372	301	238	151	101	71	52	31	19	10	3
Steatosis ≥10%	179	137	109	84	59	43	33	27	17	9	3	0

(f)



Number of patients at risk

BMI ≥23

Steatosis 0–9%	417	346	269	213	129	94	66	49	31	19	8	4
Steatosis ≥10%	226	176	137	101	71	55	34	33	20	10	5	0

BMI <23

Steatosis 0–9%	510	415	351	290	191	133	95	68	46	30	19	6
Steatosis ≥10%	126	95	70	56	42	28	20	15	12	7	4	1

Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) among patients with steatosis (solid line) and without steatosis (dotted line), stratified by other risk factors. The cumulative incidence of HCC was (a) significantly higher in patients with a steatosis grade of 10% or greater ($P < 0.0001$ by the log-rank test), even after (b) stratification by the response to interferon therapy ($P < 0.0001$ for sustained virological response [SVR] and non-SVR by the log-rank test), (c) stratification by the stage of fibrosis ($P < 0.0001$ for F0–2 and $P = 0.0036$ for F3–4 by the log-rank test), (d) stratification by age ($P = 0.0001$ for age ≥60 and $P < 0.0001$ for age <60 by the log-rank test), (e) stratification by sex ($P < 0.0001$ for men and women by the log-rank test), and (f) stratification by body mass index (BMI) ($P < 0.0001$ for BMI ≥23 kg/m^2 and <23 kg/m^2 by the log-rank test). The number of patients at risk is shown below each graph.

age, sex, HCV genotype and stage of fibrosis.[19] In this study, only ALT and albumin were identified as predictors of HCC and steatosis was not. The authors acknowledged the small size of the cohort as a limitation and emphasized the need for larger cohort studies. The third study analyzed explanted liver from cirrhotic patients who underwent liver transplantation and included 32 patients with HCC and 62 patients without HCC.[18] The authors found that older age, higher α-fetoprotein levels and steatosis were significantly associated with HCC. The major advantage of this study was the standardization of fibrosis stage to cirrhosis. On the other hand, a limitation was the retrospective nature of the study; steatosis was evaluated after the diagnosis of HCC, when cirrhosis already was present (fibrosis stage F4). Because steatosis has been reported to decrease once cirrhosis has developed, this study may have underestimated the grade of steatosis present prior to the development of HCC. Thus, we cannot simply apply their findings to a clinical setting where biopsies are usually obtained before the development of cirrhosis and years before the development of HCC. Based on that background, the principal aim of this study was to analyze the association between hepatic steatosis and the development of HCC in chronic hepatitis C patients, adjusting for known risk factors. We found that steatosis was an independent risk factor by the multivariate Cox proportional hazards regression analysis and by the Kaplan–Meier method and log-rank test after stratification by other risk factors. To our surprise, the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI.

How steatosis contributes to the development of HCC remains unclear. Several studies including ours,[10] indicated that hepatic steatosis promotes the progression of hepatic fibrosis,[11–15] which potentiates the risk of HCC indirectly. On the other hand, the ob/ob mouse model of NAFLD showed that hepatic neoplasia developed in the absence of advanced fibrosis, supporting the concept that metabolic abnormalities related to obesity initiate the neoplastic process.[8] Leptin, an adipocytokine related to steatosis in chronic hepatitis C,[21] was shown recently to be mitogenic in human liver[22] and thus may be a link between steatosis and HCC development. Otherwise, steatosis may be responsible for increased lipid peroxidation and reactive oxygen species which induce genetic damage.[23–25] Another study showed that mice transgenic for the HCV core gene developed hepatic steatosis early in life and thereafter HCC which indicates that the HCV core protein has a chief role in the development of both steatosis and HCC development.[26] The precise mechanism of the association between steatosis and carcinogenesis needs further investigation.

The higher incidence of HCC in patients with significant steatosis has important clinical implications. The most important question is whether therapeutic interventions aimed at reducing steatosis could reduce the risk of HCC in chronic hepatitis C. Because the adjusted risk ratio of hepatic steatosis was higher than that of older age, male sex, non-SVR to IFN and higher BMI, we hypothesize that modification of lifestyle and the amelioration of hepatic steatosis may efficiently prevent hepatocarcinogenesis in patients having concomitant risk factors. Apparently, further prospective studies focusing on this point are necessary. Weight reduction may provide an important treatment strategy because one study indicated that weight reduction in chronic hepatitis C leads to a reduction in steatosis and an improvement in fibrosis despite the persistence of HCV infection.[27] Alternatively, insulin resistance may be another target of therapy because a study showed that the administration of pioglitazone led to metabolic and histological improvement in subjects with non-alcoholic steatohepatitis.[28] A limitation of the present study was that data for the plasma insulin concentration was not available and thus insulin resistance could not be assessed. Whether insulin resistance plays a role in hepatocarcinogenesis or its amelioration could improve steatosis and ultimately prevent development of HCC in chronic hepatitis C awaits future investigation.

Another important finding of the present study was that steatosis was a significant risk factor for the development of HCC in patients with SVR to IFN therapy. Thus, steatosis may play a role in carcinogenesis in patients who have cleared HCV. Several studies have shown that the incidence of HCC is reduced but not eliminated in those with SVR to IFN.[29–31] Because the predictors of HCC development in SVR patients have not been established to date, steatosis may be used to identify patients who need intensive surveillance and long-term follow up, even after the clearance of HCV. In conclusion, we showed that hepatic steatosis is significantly associated with the development of HCC in chronic hepatitis C independent of age, sex, BMI, degree of fibrosis and response to previous IFN therapy. Steatosis may be a useful marker for identifying patients at higher risk for HCC. Further studies are needed to evaluate the hypothesis that therapeutic interventions aimed at reducing steatosis may prevent hepatocarcinogenesis.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Pretreatment prediction of response to peginterferon plus ribavirin therapy in genotype 1 chronic hepatitis C using data mining analysis

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Abstract
Background This study aimed to develop a model for the pre-treatment prediction of sustained virological response (SVR) to peg-interferon plus ribavirin therapy in chronic hepatitis C.
Methods Data from 800 genotype 1b chronic hepatitis C patients with high viral load (>100,000 IU/ml) treated by peg-interferon plus ribavirin at 6 hospitals in Japan were randomly assigned to a model building ($n = 506$) or an internal validation ($n = 294$). Data from 524 patients treated at 29 hospitals in Japan were used for an external validation. Factors predictive of SVR were explored using data mining analysis.
Results Age (<50 years), alpha-fetoprotein (AFP) (<8 ng/mL), platelet count ($\geq 120 \times 10^9$/l), gamma-glutamyl-transferase (GGT) (<40 IU/l), and male gender were used to build the decision tree model, which divided patients into 7 subgroups with variable rates of SVR ranging from 22 to 77%. The reproducibility of the model was confirmed by the internal and external validation ($r^2 = 0.92$ and 0.93, respectively). When reconstructed into 3 groups, the rate of SVR was 75% for the high probability group, 44% for the intermediate probability group and 23% for the low probability group. Poor adherence to drugs lowered the rate of SVR in the low probability group, but not in the high probability group.

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Conclusions A decision tree model that includes age, gender, AFP, platelet counts, and GGT is useful for predicting the probability of response to therapy with peg-interferon plus ribavirin and has the potential to support clinical decisions regarding the selection of patients for therapy.

Keywords Data mining · Decision tree · Alpha-fetoprotein · HCV · Peg-interferon

Introduction

The current standard therapy for genotype 1 chronic hepatitis C is 48 weeks of pegylated interferon (PEG-IFN) plus ribavirin (RBV) [1]. Sustained virological response (SVR), defined as undetectable HCVRNA post-treatment is regarded as a cure of chronic hepatitis C. However, the rate of SVR to this regimen is only 50% in patients with HCV genotype 1b and a high HCVRNA titer [2, 3]. Since PEG-IFN and RBV combination therapy is costly and accompanied by potential adverse effects, the ability to predict the possibility of SVR before therapy may significantly influence the selection of patients for therapy. A recent report revealed that single nucleotide polymorphisms located in the *IL28B* are strongly associated with a response to PEG-IFN plus RBV therapy [4–6]. Besides, the amino acid substitutions in the NS5A [7–9] or core region of HCV were also associated with response to therapy [10, 11]. Unfortunately, these host genetic and viral factors are not yet readily available for general application in actual clinical practice. Fibrosis of the liver is also an important predictor of response, but resources may be limited in some countries. Clinical and non-invasive parameters may be better suited for general practice, but there is no established means by which the likelihood of a response can be predicted prior to therapy.

Data mining is a method of predictive analysis that explores data, without setting the hypothesis, to discover hidden patterns and relationships in highly complex datasets and enables the development of predictive models. Decision tree analysis is a core component of data mining and predictive modeling [12], and it is utilized by decision makers in various fields of business. Recent publications on decision tree analysis indicate its usefulness for defining prognostic factors in various diseases such as prostate cancer [13], diabetes [14], melanoma [15, 16], colorectal carcinoma [17, 18], and liver failure [19]. The results of the analysis are presented as a tree structure, which is intuitive and facilitates the allocation of patients into subgroups by following the flow chart form [20]. We have recently reported the usefulness of decision tree analysis for the prediction of early virological response (undetectable HCVRNA within 12 weeks of therapy) to PEG-IFN and RBV combination therapy in chronic hepatitis C [21].

In the present study, we used decision tree analysis to explore baseline predictors of response to PEG-IFN/RBV therapy so that a pre-treatment algorithm could be created to discriminate chronic hepatitis C patients who are likely to respond to PEG-IFN/RBV therapy from those who are not. For the purpose of use in general practice, only clinical and non-invasive parameters were included in the analysis.

Materials and methods

Patients

This was a multicenter retrospective cohort study supported by the Japanese Ministry of Health, Labor and Welfare. Data were collected from a total of 800 chronic hepatitis C patients who received therapy for 48 weeks with PEG-IFN alpha-2b and RBV at Musashino Red Cross Hospital, Toranomon Hospital, Tokyo Medical and Dental University, Osaka University, Nagoya City University Graduate School of Medical Sciences, Yamanashi University, and their related hospitals. The inclusion criteria to be enrolled in this study were as follows (1) infection by genotype 1b, (2) HCVRNA higher than 100,000 IU/ml by quantitative PCR (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic systems, CA), which is typically used for the definition of high viral load in Japan, (3) lack of co-infection with hepatitis B virus or human immunodeficiency virus, (4) lack of other causes of liver disease such as autoimmune hepatitis and primary biliary cirrhosis and (5) completion of at least 12 weeks of therapy. Patients received PEG-IFN alpha-2b (1.5 μg/kg) subcutaneously every week and were administered a weight-adjusted dose of RBV (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg), which is the recommended dosage in Japan. Patients who were treated for more than 49 weeks were not included in the study. For the analysis, patients were randomly assigned to either the model building ($n = 506$) or the internal validation ($n = 295$) group. Consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committee. The baseline characteristics and representative laboratory test results are listed in Table 1. The overall rate of SVR was 47% in the model building set and 49% in the validation set. There were no significant differences in the clinical backgrounds between these 2 groups.

For external validation of the model, we collaborated with another study group supported by the Japanese Ministry of Health, Labor and Welfare. This multicenter study group consisted of 29 medical centers and hospitals

Table 1 Comparison of pre-treatment factors between model building and internal validation patients

	Model ($n = 506$)	Validation ($n = 295$)
Age (years)	56 (14–75)	55 (18–74)
Male gender[a]	261/506 (52%)	160/295 (54%)
Body mass index (kg/m^2)	22.9 (14.3–34.0)	23.2 (16.1–33.8)
Albumin (g/dl)	4 (2.7–5.0)	4 (2.8–4.9)
Creatinine (mg/dl)	0.7 (0.4–1.5)	0.7 (0.4–1.1)
AST (IU/l)	60 (11–370)	62 (11–240)
ALT (IU/l)	73 (11–413)	73 (14–390)
GGT (IU/l)	56 (10–328)	55 (7–409)
Total cholesterol (mg/dl)	173 (73–297)	171 (29–273)
Triglyceride (mg/dl)	105 (33–474)	109 (32–372)
White blood cell count (/μl)	4,745 (1,800–10,900)	4,823 (1,200–9,700)
Neutrophil count (/μl)	2,563 (667–7,870)	2,484 (508–7,579)
Red blood cell count (/μl)	448 (313–577)	451 (313–574)
Hemoglobin (g/dl)	14.1 (9.4–18.3)	14.1 (10.0–18.0)
Hematocrit (%)	41.7 (13.3–53.7)	41.9 (15.5–52.7)
Platelets (10^9/l)	164 (52–380)	158 (43–312)
AFP (ng/ml)	14.7 (0.9–680)	13 (0.8–323)
HCVRNA (10^3 IU/ml)	1,852 (100–5,100)	1,870 (100–5,100)
Fibrosis stage: F3–4	73/417 (18%)	48/247 (19%)

Data expressed as median (range) unless otherwise indicated

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma-glutamyltransferase, *AFP* alpha-fetoprotein

[a] Data expressed as number/available data (percentage)

belonging to the National Hospital Organization. A dataset collected from 524 patients who were treated with PEG-IFN alpha-2b/RBV was used as an external validation dataset, i.e., completely independent from the dataset that was used for model building.

Laboratory tests

Blood samples were obtained before therapy and at least once every month during therapy, and were used for hematologic tests, blood chemistry analysis and determination of HCV RNA. Pretreatment levels of HCV RNA were quantified by Cobas Amplicor (Roche Diagnostic Systems, Pleasanton, CA). SVR was defined as undetectable HCV RNA at week 24 after completion of therapy, as determined by qualitative PCR with a lower end detection limit of 50 IU/ml (Amplicor, Roche Diagnostic Systems). Liver biopsy was available in 664 patients. Fibrosis and activity were scored according to the METAVIR scoring system [22]. Fibrosis was staged on a scale of 0–4: F0 (no fibrosis), F1 (mild fibrosis: portal fibrosis without septa), F2 (moderate fibrosis: few septa), F3 (severe fibrosis: numerous septa without cirrhosis) and F4 (cirrhosis). Activity of necroinflammation was graded on a scale of 0–3: A0 (no activity), A1 (mild activity), A2 (moderate activity) and A3 (severe activity).

Statistical analysis

A database of pretreatment variables was created containing 6 variables from hematological tests (red blood cells, hemoglobin, hematocrit, white blood cells, neutrocytes and platelets), 8 variables from the blood chemistry test [creatinine, albumin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase (GGT), total cholesterol, triglyceride and alpha-fetoprotein (AFP)], serum level of HCV RNA and 3 variables for patient characteristics (age, gender and body mass index). Based on this database, the recursive partitioning analysis algorithm referred to as decision tree analysis was implemented to define meaningful subgroups of patients with respect to the possibility of achieving SVR.

Decision tree analysis is a family of nonparametric regression methods. Software is used to automatically explore the data to search for optimal split variables and to build a decision tree structure [23]. For the analysis, the entire study population was evaluated to determine which variables and cutoff points yielded the most significant division into 2 prognostic subgroups that were as homogeneous as possible for the probability of SVR. Thereafter, the same analytic process was applied to all newly defined subgroups. A restriction was imposed on the tree construction such that the procedure stopped when either no additional significant variable was detected or when the sample size was below 20. For this analysis, the data mining software IBM SPSS Modeler 13 (IBM SPSS Inc., Chicago, IL) was utilized. SPSS software v.15.0 (SPSS Inc., Chicago, IL) was used for multivariate logistic regression analysis.

Results

Decision tree analysis

Decision tree analysis was carried out on the model building dataset from 506 patients using 18 variables. Figure 1 shows the results. The analysis automatically selected 5 predictive variables to produce a total of 7 subgroups of patients. Age was selected as the variable of initial split with an optimal cutoff of 50 years. The possibility of achieving SVR was 41% for patients older than 50 compared to 70% for patients

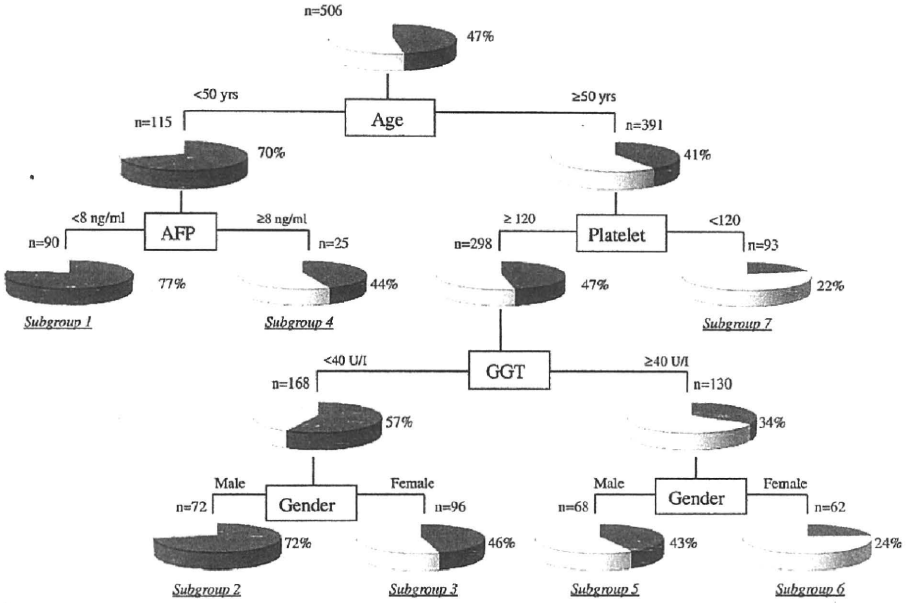

Fig. 1 Decision tree analysis. *Boxes* indicate the factors for splitting and the cutoff value for the split. *Pie charts* indicate the rate of SVR for each group. Terminal subgroups of patients discriminated by the analysis are numbered from 1 to 7. *AFP* alpha-fetoprotein, *GGT* gamma-glutamyltransferase

younger than 50. Among patients younger than 50, the level of serum AFP, with an optimal cutoff of 8 ng/ml, was selected as the variable of second split. Patients with lower AFP levels had a higher probability of SVR (77 vs. 44%). Among older patients, platelet count was selected as the second variable of split, with an optimal cutoff of 120×10^9/l. Patients with higher platelet counts had a higher probability of SVR (47 vs. 22%). Among patients with platelet counts higher than 120×10^9/l, GGT was selected as the third variable of split with an optimal cutoff of 40 IU/l. Patients with a lower GGT level had a higher probability of SVR (57 vs. 34%). Gender was selected as the fourth variable of split, with male gender being a predictor of a higher SVR probability (72 vs. 46% in patients with GGT levels <40 IU/l and 43 vs. 24% in those with GGT ≥40 IU/l). HCVRNA load was included in the analysis but was not selected as a significant variable.

The probabilities of SVR for the 7 subgroups derived by this process were highly variable. The subgroup of young patients (<50 years) with low serum AFP (<8 ng/ml) (subgroup 1) or the subgroup of older (≥50 years) male patients with high platelet counts ($\geq 120 \times 10^9$/l) and low serum GGT (<40 IU/l) (subgroup 2) showed the highest probability of SVR (72 and 77%), while the subgroup of older (≥50 years) patients with low platelet counts ($<120 \times 10^9$/l) (subgroup 7) and older (≥50 years) female patients with high serum GGT (subgroup 6) showed the lowest probability of SVR (22 and 24%).

Validation of the decision tree

The results of the decision tree analysis were validated with an internal validation dataset of 295 cases, which was independent of the model building dataset. Each patient in the validation set was allocated to subgroups 1–7 using the flow-chart form of the decision tree. The rates of SVR were 77% for subgroup 1, 71% for subgroup 2, 55% for subgroup 3, 44% for subgroup 4, 41% for subgroup 5, 17% for subgroup 6, and 30% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated between the model building dataset and the internal validation dataset ($r^2 = 0.925$) (Fig. 2a).

To further confirm the universality of the results, data collected from 524 patients by a collaborating study group were used for external validation. Thus, the dataset used for external validation was completely independent of the

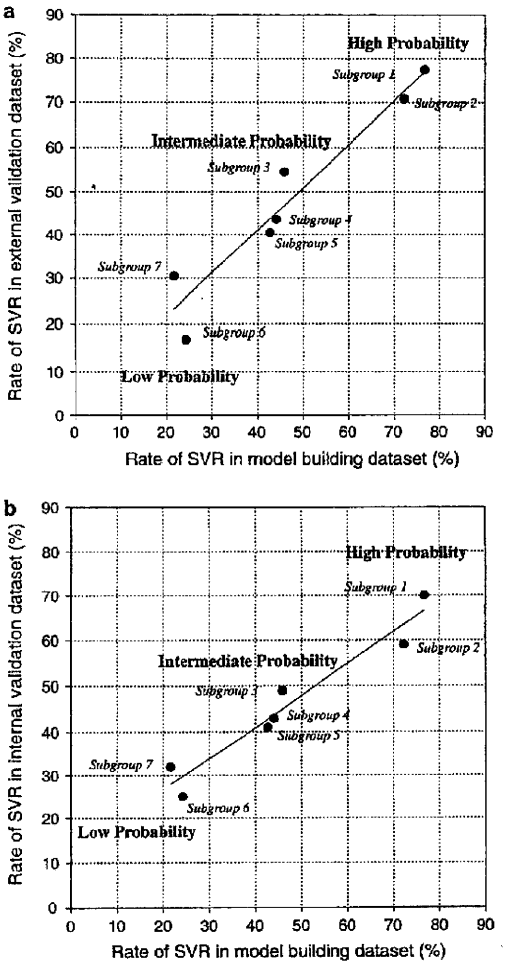

Fig. 2 Validation of the decision tree analysis by an internal and external validation dataset: subgroup-stratified comparison of the SVR rate. The rate of SVR in each subgroup was plotted. The *X* axis represents the model building, and the *Y* axis represents the validation datasets. **a** Internal validation and **b** external validation. There was a close correlation between the model building and the internal validation dataset (correlation coefficient $r^2 = 0.925$) and between the model building and the external validation dataset (correlation coefficient $r^2 = 0.936$)

original dataset used for model building. Each patient in the external validation set was allocated to subgroups 1–7 using the flow-chart form of the tree. The rates of SVR were 70% for subgroup 1, 59% for subgroup 2, 49% for subgroup 3, 43% for subgroup 4, 41% for subgroup 5, 25% for subgroup 6, and 32% for subgroup 7. The rates of SVR for each subgroup of patients were closely correlated

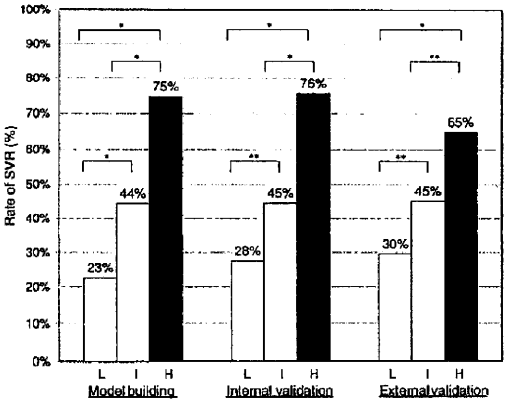

Fig. 3 Comparison of SVR rates between groups divided by the decision tree. The rate of SVR was compared among the 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes*, indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively). The rate of SVR was significantly different among the 3 groups. $*p < 0.0001$, $**p < 0.001$

between the model-building dataset and the validation dataset ($r^2 = 0.936$) (Fig. 2b).

Construction of 3 groups according to the probability of SVR

Seven subgroups were reconstructed into 3 groups according to their predicted rates of SVR: the high probability group consisted of subgroups 1 and 2, the intermediate probability group consisted of subgroups 3, 4 and 5, and the low probability group consisted of subgroups 6 and 7. The rate of SVR was significantly different among the 3 groups (Fig. 3). The rate of SVR in the high probability group was consistently high: 75% for model building patients, 76% for internal validation patients and 65% for external validation patients. Conversely, the rate of SVR in the low probability group was consistently low: 23% for model building patients, 28% for internal validation patients and 30% for external validation patients. The rate of SVR in the intermediate probability group was 44% for model building patients, 45% for internal validation patients and 45% for external validation patients. Since 28–32% of patients were classified as high probability and 30–32% were classified as low probability, roughly 60% of patients were classified as having either a high or low probability of achieving SVR.

Effect of dose reductions of PEG-IFN and RBV on SVR

The cumulative dose of PEG-IFN and RBV was not included as a variable of analysis since the present study

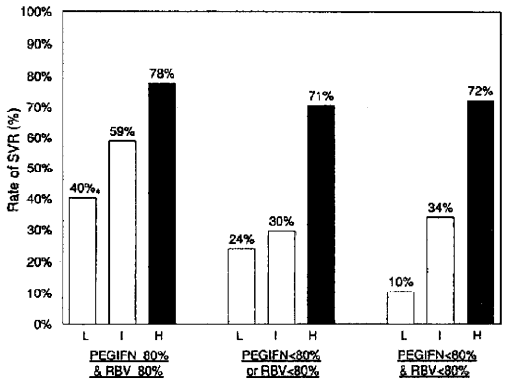

Fig. 4 Comparison of SVR rates among groups stratified by drug adherence. The 3 groups of patients divided by the decision tree analysis (*white*, *gray* and *black boxes* indicating a low (*L*), intermediate (*I*) and high (*H*) probability group, respectively) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV. The good adherence group (≥80% planned dose of both PEG-IFN and RBV) had a higher rate of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) but not in the high probability group ($p = 0.53$)

aimed to develop a pre-treatment model for the prediction of response. To analyze the possible effect of drug reductions on the result of the decision tree analysis, 3 groups of patients divided by the decision tree analysis (low, intermediate and high probability group) were further stratified according to the cumulative drug exposure of PEG-IFN and RBV (Fig. 4). Even after adjustment for adherence, 3 groups of patients still had low, intermediate and high probability of achieving SVR, respectively. Of note, the good adherence group (≥80% planned dose of both PEG-IFN and RBV) had higher rates of SVR compared with the poor adherence group (<80% planned dose of both PEG-IFN and RBV) in the low ($p = 0.0003$) and intermediate ($p = 0.007$) probability group, but not in the high probability group ($p = 0.53$).

Factors associated with SVR by multivariate logistic regression analysis

We also explored the factors associated with SVR using a standard statistical analysis. By univariate analysis, age, gender, serum albumin, creatinine, alanine aminotransferase, GGT, red blood cell count, hemoglobin, hematocrit, platelet count and AFP were found to be associated with SVR (Table 2). HCVRNA load was not associated with SVR. By multivariate analysis, age, gender, GGT and platelet count were found to be independently associated with SVR (Table 3). Of note, AFP, which was selected as a significant predictor of response in the decision tree analysis, was not found to be an independent response predictor in the standard multivariate analysis. This indicates a unique feature of the decision tree analysis; i.e., it could identify significant predictors that specifically apply to selected patients, in this case patients younger than 50 years old.

Relationships between decision tree model and stage of fibrosis or HCV RNA load

Liver biopsy was performed in 664 patients. The distribution of fibrosis in three probability groups differed significantly. Advanced fibrosis (F3 or F4) was higher in the low probability group (39%) compared to the intermediate probability group (13%) ($p < 0.0001$) and to the high probability group (6%) ($p < 0.0001$). Advanced fibrosis was also higher in the intermediate group compared to the high probability group ($p = 0.01$). AFP was significantly associated with liver fibrosis stage: medians of AFP levels were 4.9, 5.9, 13.0 and 18.6 for F1, F2, F3 and F4, respectively ($p < 0.0001$, Spearman's rank correlations). Lower platelet counts correlated with advanced fibrosis stages (data not shown). The SVR rate was higher in the high probability group compared to the intermediate or low probability group after stratification by HCV RNA load. Among patients with low HCVRNA load (<400,000 IU/ml), the rate of SVR was 93, 59 and 50% for the high, intermediate and low probability group, respectively ($p = 0.002$ for high vs. intermediate and $p < 0.001$ for high vs. low probability groups). Among patients with a high HCVRNA load (≥400,000 IU/ml), the rate of SVR was 73, 42 and 21% for the high, intermediate and low probability group, respectively ($p < 0.001$ for high vs. low, high vs. intermediate and intermediate vs. low probability groups).

Discussion

Currently, the combination of PEG-IFN and RBV is the recommended therapy for chronic HCV infection. The rate of SVR with 48 weeks of therapy is around 50% in patients with HCV genotype 1b and a high HCV RNA titer [2, 3]. To date, the virological response during therapy is the most reliable means for predicting the likelihood of SVR [2, 24, 25]. More potent therapy, such as a triple combination of protease inhibitor, PEG-IFN and RBV, is being evaluated in clinical trials but is not readily available [26, 27]. Under the circumstances, pre-treatment prediction of the likelihood of SVR may be useful for both patients and physicians to support clinical decisions as to whether to start PEG-IFN/RBV therapy or delay treatment until a new more effective therapy becomes available.